DETERMINING THE EFFECTS OF LIVESTOCK GRAZING ON YOSEMITE TOADS (*BUFO CANORUS*) AND THEIR HABITAT: AN ADAPTIVE MANAGEMENT STUDY

Final Study Plan – 14 September 2007

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PROBLEM REFERENCE AND LITERATURE

Introduction

Since the late 1970s, scientists have discovered an alarming decrease in amphibian populations around the world (Stuart et al. 2004, Houlahan et al. 2000). A recent review of amphibian declines identified six potential causes: land use change, introductions of alien species, over-exploitation, pesticides and other toxins, infectious disease, and global change, which includes both increased ultraviolet (UV) radiation and climatic change (Collins and Storfer 2003). Climate change may be particularly detrimental to amphibians because they are sensitive to subtle differences in temperature as well as timing of precipitation and snow events (Beebee 1995). In some environments, trampling of riparian areas, reduction of plant species diversity, and water quality degradation caused by improper livestock grazing are also potential causes of amphibian species declines (Fleischner 1994).

Amphibian species are declining in montane areas, where anthropogenic impact is seemingly low compared to other landscapes (Wake and Morowitz 1991). Recent research has demonstrated that windborne pesticides/toxins and global change are potential factors in these settings (Davidson et al. 2002, Carey and Alexander 2003, Sparling and Cowman 2003). Recent bioregional assessments and land management plans for the Sierra Nevada mountain range have identified declining species and provided recommendations and standards for reducing impacts to these species (Sierra Nevada Ecosystem Project 1996, USDA Forest Service 2001, 2004). Almost half of the 30 or so native amphibian species in the Sierra Nevada are considered to be at risk by State and Federal regulatory agencies (Jennings and Hayes 1994, California Department of Fish and Game 2004, USDI Fish and Wildlife Service 2002, 2004). This mountain range comprises only 20% of the total land area of California, yet 50% of the native plant species in the state occur within it. Over 3,500 plant species, 400 of which are endemic, occur in the Sierra Nevada (D'Antonio et al. 2002). Within this range, montane meadows are areas of high biodiversity, supporting many native and rare species, providing forage and water for grazing animals, and offering a distinct habitat contrast with surrounding areas (Allen-Diaz 1991, Gavin and Brubaker 1999, Ratliff 1985). Meadows comprise less than 10% and riparian areas are less than 1% of the Sierra Nevada, but their ecological importance is disproportionate to their size (Ratliff 1985, Kattleman and Embury 1996).

Yosemite Toads

The Yosemite toad (*Bufo canorus*) is endemic to the Sierra Nevada mountain range from the Blue Lakes region north of Ebbetts Pass in Alpine County south to Kaiser Pass area in the Evolution Lake/Darwin Canyon region of Fresno Co at elevations from ca. 1950 m ca. 3600m (6400 to 11,800 ft). Yosemite toads are typically associated with high montane and subalpine vegetation in relatively open wet meadows surrounded by forests of lodgepole pine or whitebark pines and are primarily active during the late spring, summer, and early fall (Zeiner et. al. 1988, Jennings and Hayes 1994). Adult females are slightly larger (69 versus 66 mm) and heavier (20 versus 17 grams) than adult males. Males and females vary markedly in color; males are olive green while females are gray or brown (Kagarise Sherman and Morton 1984). Cover requirements differ by life history stage. Suitable breeding sites are generally found in shallow, warm water areas such as those found in wet meadows, potholes, the edges of small ponds, shallow, grassy areas adjacent to lakes, slow moving streams, sloughs and backwaters (C. Brown, personal communication). Short emergent sedges or rushes often dominate such sites (Jennings and Hayes 1994). Yosemite toads breed in late spring, usually at snowmelt, laying eggs in shallow areas of lakes and wet meadows; larvae metamorphose by mid-late summer of the same year. Breeding can last up to five weeks for males, while females often only visit breeding sites for two to three days. Breeding is often 1-2 weeks, but can last up to 5 weeks for males; females often only visit breeding sites for 2-3 days (Kagarise Sherman and Morton 1993, Sadinsky 2004, C. Brown, personal communication). Meadow water depth and water temperature appear to be important limiting factors in the survival of eggs and larvae (Kagarise Sherman and Morton 1993). These characteristics are strongly influenced by winter snow pack, spring temperatures, and meadow topography/hydrology, but direct relationships to toad habitat use and egg/larval survival have not been quantified. Species success is heavily dependant on weather, with variations in climate such as changing storm patterns and drought delaying or interrupting breeding and affecting survival of metamorphic toads and adults (Kagarise Sherman and Morton 1993, Jennings and Hayes 1994). Adults utilize aquatic and terrestrial environments for foraging and cover. Springs, upslope from meadows, and rodent burrows are two features that appear to be important for adult dispersal and over wintering habitat (Kagarise Sherman 1980).

Yosemite toads are believed to have declined or disappeared from at least 50% of known localities during the later part of the 20th century (Jennings and Hayes 1994, Drost and Fellers 1996, Jennings 1996) though limited quantitative surveys exist. Long-term monitoring data at Tioga Pass indicate large declines in local populations since the early 1980's (Kagarise Sherman and Morton 1993), although the cause for this decline is unclear. Yosemite toads are a Species of Special Concern in California, a Forest Service Region 5 sensitive species, and a candidate species for federal listing under the Endangered Species Act (USDI Fish and Wildlife Service 2002, 2004). Potential factors that could individually or collectively be responsible for the decline of Yosemite toads include livestock grazing in montane meadows and riparian zones, airborne chemical toxins, disease, and climatic shifts and variability, though none of these has emerged as a singularly strong candidate (Davidson et al. 2002, USDI Fish and Wildlife Service 2002). Research seems to be eliminating possibilities instead of verifying definite causes. For example, an extensive scale study by Knapp (2005) concluded that introduced trout are not a major concern for Yosemite toads. In a predation experiment, Grasso (2005) found that toad larvae and post-metamorphic young of year were unpalatable to common introduced trout species. Another study by Bradford et al. (1994) found no correlation between acid deposition and Yosemite toad decline, and suggested that disease is potentially the greatest cause of decreased numbers. Two diseases that may affect Yosemite toad populations are red-legged disease and chytrid fungus (USDI Fish and Wildlife Service 2002).

Of the activities occurring on National Forest lands and under the jurisdiction of Forest Service management, livestock and packstock grazing have been identified as activities that may affect Yosemite toads (USDA Forest Service 2001). This is because of the overlap of grazing with toad breeding and rearing areas in wet meadows. Though cattle are rarely present during the brief breeding period at snow-melt, they are often present during larval rearing and metamorphosis. While this potential risk factor has been identified in the public record, the supporting data are primarily from anecdotal accounts and unpublished sources (USDI Fish and Wildlife Service 2002). Preliminary evidence suggests that livestock use of wet meadow habitats may affect Yosemite toads indirectly through: (1) changes to meadow stream hydrology and bank stability (increased down-cutting and head-cutting), (2) changes to water quality, and (3) changes in micro-topography of egg deposition and larval rearing areas (USDI Fish and Wildlife Service 2002). The extent of these impacts and their relationship to population level survival and persistence need further study. Due to a reported Yosemite toad population decline and its suspected link to livestock grazing, the USFS placed in non-use a number of previously utilized Sierra Nevada grazing permits in 2001. Many of these allotments had been active for over one hundred and fifty years (USDA Forest Service 2001). A study on this subject is urgently needed both to provide guidance to land managers who are faced with decisions regarding human and livestock use of montane meadows and to better understand the role that livestock grazing may be playing in the decline of the Yosemite toad.

Livestock Grazing

The Spanish introduced livestock to the Sierra Nevada in the mid-1700s, but extensive grazing did not occur until the 1860s (Menke et al. 1996). This was due in part to population increase following the Gold Rush. Drought and flooding also pushed ranchers to higher elevations in the Sierra Nevada during that time. Unregulated and non-sustainable grazing practices were common into the late 1800s (Menke et al. 1996). The establishment of Forest Reserves and subsequently the Forest Service (1905) alleviated the situation by instituting a grazing management policy (Kinney 1996). The Taylor Grazing Act (1934) also led to the implementation of federal land policies and helped end the "open access" era of grazing in the Sierra Nevada (Allen-Diaz et al. 1999). Stocking rates have fluctuated some since then, but have continued to gradually decline (Allen-Diaz et al. 1999, Kosco and Bartolome 1981). The issuance of fewer permits has widespread social (e.g. forcing ranchers to sell their operations) and ecological implications and is a common topic in research and management discussion (Sulak and Huntsinger 2002).

In addition to the subject of permits, other aspects of grazing are often polarized, misunderstood, and/or ambiguous (Brown and McDonald 1995). Researchers and managers frequently identify grazing as either good or bad without consideration of the intensity, timing, frequency, duration, and season of use (Allen-Diaz et al. 1999, Fleischner 1994). Grazing treatments are also often vague, with studies citing "heavy" and "light" levels instead of outlining quantitative intensities (Trimble and Mendel 1995, Tate et al. 1999). To compound the complexity, grazing may also simultaneously negatively and positively impact different aspects of a single system (Hayes and Holl 2003, Allen-Diaz et al. 2004).

Grazing and Ecosystem Interactions

Improper grazing can negatively affect riparian and aquatic systems in a number of ways (Flenniken et al. 2001). Grazing in riparian areas may cause soil compaction, removal of vegetation, physical damage and reduction of vegetation, and alteration of plant growth forms by removal of terminal buds and stimulation of lateral branching (Kauffman and Krueger 1984, Tucker Shulz and Leininger 1990, Szaro 1989). Decreased infiltration, increased erosion and runoff, and increased soil temperature can result from these impacts (Armour et al. 1991). Grazing may change aquatic systems through degrading channel morphology, lowering the groundwater table, decreasing stream flow, altering timing and rate of flood events, and increasing stream temperature (Armour et al. 1994, Kauffman and Krueger 1984). Changes in water quality, such as increased nitrate, phosphate, dissolved solids, and sediment are additional concerns (Kauffman and Krueger 1984).

Grazing is often negatively implicated in other ecosystem changes. A study of pack stock grazing in Yosemite National Park recently found a decrease in plant productivity, a decrease in vegetation cover, and an increase in bare soil cover with increased animal utilization (Cole et al. 2004). Pack stock and cattle can also transport invasive seeds through fur and dung and open habitat for non-native species (Trimble and Mendel 1995). Preliminary assessments indicate that livestock may trample amphibian eggs, larvae, juveniles, and burrows used by adults for hibernation and cover (Jennings 1996, USDI Fish and Wildlife Service 2002).

A number of studies have conversely shown either no effect or a beneficial impact of livestock. For example, appropriate cattle grazing reduces non-native annual grasses around vernal pool margins, benefiting these fragile systems and the organisms that depend upon them (Robins and Vollmar 2002). Hayes and Holl (2003) identified mixed effects of grazing in coastal California grasslands. They found an increase in species richness and cover of native perennial forbs in ungrazed sites, but no difference in native grass cover and species richness between grazed and ungrazed sites. They concluded that a matrix of disturbance regimes is necessary to maintain a suite of native species. A study by Hickman et al. (2004) in tallgrass prairie found higher species diversity and richness in grazed versus ungrazed exclosures. An additional study by Bull and Hayes (2000) concluded that grazing did not negatively impact reproduction and recruitment of the Columbia Spotted Frog (*Rana luteiventris*).

The complexities of species decline, the social and ecological implications of different management decisions, and interactions of livestock with ecosystems are impressive. Ascertaining how to approach these issues is even more challenging. However, we do know that global biodiversity loss is occurring at an accelerated rate, and the complete implications of this decline for society and the environment are unknown (Myers et al. 2000, Forester and Machlis 1996). An array of habitats and species provide cures for human diseases, economically valuable goods and services such as clean air and water, protection from floods and droughts, and recreational opportunities (Allen-Diaz 2000, Spear 2000). As biodiversity declines and species become extinct, we lose knowledge about possible cures for human illnesses, evolutionary connections between organisms, and an understanding of ecosystem processes. Precluding species decline is therefore imperative for human well being in the future. Understanding relationships between species and their habitats and assessing how humans affect these systems in both positive and negative ways will provide the information needed for management and restoration. Our proposed study of Yosemite toads and livestock grazing will provide the knowledge needed for the management and conservation of this species and the unique environments it inhabits.

OBJECTIVES

The overall objective of this study is to understand the effects of varying levels of livestock grazing on Yosemite toad populations and habitats. USDA Forest Service Region 5 staff formulated two key questions to guide this research:

1. Does livestock grazing under Forest/Sierra Nevada Forest Plan Amendment Riparian Standards and Guidelines have a measurable effect on Yosemite toad populations?

Assessment of grazing standard and guideline variables includes monitoring meadows occupied by Yosemite toads under the following treatments:
* Ungrazed meadows (not grazed within recent history, likely outside of an active allotment).
* No grazing within the meadow (i.e. these are meadows that have been grazed recently; they will be fenced to exclude grazing during the study).
* Exclusion of livestock in wet areas within a meadow (S&G 53).
* Grazing in accordance with Riparian S&Gs 103, 120, 121 across the entire meadow.

2. What are the effects of livestock grazing intensity on the key habitat components that affect survival and recruitment of Yosemite toad populations? Key meadow habitat components include hydrology, topography, and cover.

The research proposed here will address these questions and determine whether different treatments result in different population levels and habitat conditions for Yosemite toads. This information can then be used to provide guidance to land managers on relative risk of a set of grazing (or non-grazing) approaches to the long-term survival of Yosemite toads on Forest Service lands. This latter step will require an assessment of the viability of the toad at local and more extensive spatial scales. The data we collect can be used in such an assessment, but additional data on the distribution of Yosemite toads on Forest Service lands will also be needed. Distributional data has been collected at varying degrees of detail over the last few years on the Stanislaus, Sierra, and Inyo National Forests both for range-wide long-term monitoring (C. Brown, pers. comm.) and Forest level survey requirements (S. Holdeman, H. Sanders, pers. comm.). The results of this work will also contribute to an on-going conservation assessment for the Yosemite toad and to any future conservation strategies.

STUDY AREA

The broad study area for this project is the geographic range of the Yosemite toad which includes populations on the El Dorado, Inyo, Stanislaus, Sierra, and Toiyabe National Forests and Yosemite and Sequoia Kings Canyon National Parks. Of these areas, toad populations are most abundant on the Inyo, Stanislaus, and Sierra National Forests and in Yosemite National Park (USDI Fish and Wildlife Service 2002). Because this study specifically deals with livestock grazing and this use is more prevalent in some areas, the focal areas for meadow selection include the Stanislaus and Sierra National Forests (livestock treatment meadows and possible reference meadows) and potentially Yosemite National Park (reference meadows).

METHODS AND ANALYSIS

Study Design

Overview

The project will be based upon two complementary study designs (described here as Phases I and II) both focused on achieving the established study objectives. The experimental unit for each phase is an individual meadow. The population of interest is meadows in the central Sierra Nevada between 1829 and 3048m (6000 and 10,000 feet) in elevation which currently support a Yosemite toad population and/or have the potential to support a toad population. Inherent meadow to meadow variability (hydrology, geomorphology, disturbance regime, etc.) combined with the complexities of conducting research at the management scale make strict application of experimental methods (treatment, control, replication, complete randomization) difficult. For instance, no two meadows have replicate biology, ecology, hydrology, geology, and disturbance regimes. A combination of experimental and observational methods will be applied with heavy reliance upon multivariate statistical procedures to: 1) identify and quantify general associations between cattle grazing management, critical toad habitat factors, and toad population dynamics with quantitative evaluation of if and how these relationships are conditioned by meadow specific factors/covariates (Phase I) and 2) specifically test the effects of 3 grazing treatments on toad populations and habitat (Phase II). The design for each phase is described in detail in the following sections, as are the strengths and weaknesses of each design.

Phase I

Approach

Phase I will be an observational, cross-sectional survey of a larger set of meadows (n>50) which will be sub-sampled from the population of interest. Cattle grazing management will not be manipulated on-the-ground; rather we will take opportunistic advantage of the diversity of grazing management which we know exists across the population of meadows of interest (defined above). Previous grazing management surveys in the region (Ward et al., 2001) confirm that significant gradients exists for management factors such as: 1) grazing intensity, frequency, and season; 2) grazing distribution practices (*e.g.*, none, herding effort, drift fencing); 3) proximity to cattle attractants (*e.g.*, significant forage resources, drinking water), and 4) exclusion (*e.g.*, 1, 10, 20 years of exclusion). The assumption inherent in this approach is that toad population and habitat metrics at a point in time integrate recent grazing management (last 5 to 10 years), and are sensitive to variation in grazing impact levels. Similar assumptions have been accepted and widely applied in development and application of bioassessment protocols such as those based on aquatic macroinvertebrate community composition (Wohl et al., 1996; Hawkins et al., 2000; Weigel et al., 2000).

This design is based upon the quantification (directly measure) and/or classification (assign to a category such as "low" or "high") of toad population, habitat, vegetation, and cattle grazing variables. Multivariate analysis of these variables in conjunction with covariates (*e.g.*, elevation, meadow size) is then used to identify and quantify associations between toad population, habitat, and specific grazing management factors dependent upon site specific conditions. Significant variation can be expected across the population of meadows of interest for all factors of interest. It can also be expected that factors will potentially interact to condition toad response to grazing. It is crucial that the sub-sample of meadows selected for this survey: 1) are proportionately representative of the range of conditions (elevation, disturbance regime, etc.) found in the population of interest; 2) represent the complete gradient of variables of specific interest (toad population, habitat, grazing management) found across the population; 3) have sufficient overlap in levels of variables of interest to allow examination of potentially important interactions between these variables (*e.g.*, grazing frequency by meadow size interaction might describe the effect of grazing frequency upon toad presence or absence). We will not be able to determine adequate sample size for this phase until we have compiled a sufficient sub-sample for preliminary analysis. We were able to identify and quantify relationships between grazing and riparian health metrics in mountain meadows via a cross-sectional survey that enrolled ~60 Sierra Nevada meadows (Ward et al., 2001).

As a first step, existing data on toad population, habitat, vegetation, grazing management, and meadow covariates will be capitalized upon. It is crucial that the data collected across meadows be comparable in terms of methodology, collection date/season, units, etc. This will certainly be an issue in utilizing existing datasets. However, it is reasonable to expect that there will be reliable metrics common among datasets for toads, habitat, and vegetation, respectively. For instance, the simple metric of toad presence (yes, no) can almost certainly be derived from existing datasets with relative certainty of accuracy and comparability across meadows. However, number of adult toads per meadow is a metric that will most likely not be consistently available or credible for all datasets.

We will limit our use of existing datasets to those which were collected as part of a wide-spread, consistent survey or data collection effort with clear quality control measures in place. Such data are known to exist in the region for both toads and vegetation (Personal Communications: C. Brown, H. Sanders, and S. Holdeman, USDA Forest Service Stanislaus and Sierra National Forests, C. Miliron, California Department of Fish and Game). Overlap of these datasets across a sufficiently large and diverse sub-set of meadows will be an important factor in determining the feasibility of using only existing data. Strategic field data collection will occur during year 1 if augmentation and/or confirmation of existing datasets is required. Grazing data will certainly need to be collected via interviews with grazing managers, permit holders, and district staff. We have a cattle management survey available for this purpose which was developed and used extensively in a similar previous project.

Statistical Analysis

Phase I will generate data on several dependent or response variables, requiring multiple analyses (development of statistical models for each response variable). Dependent variables available in this phase will be toad population (*e.g.*, presence or absence, density of breeding areas per meadow) and toad habitat/vegetation (e.g., number and condition of breeding areas, meadow hydrology measures, emergent vegetation) metrics which are both categorical and continuous. Independent variables will include grazing management metrics (*e.g.*, season of grazing, stocking rate, herding effort as days/year). Covariate data from each meadow will be available for a suite of factors (*e.g.*, elevation, pack-stock use levels, and mean annual snowfall).

We have had success in modeling similar survey datasets using linear models, linear mixed effects analysis, and logistic regression models (Ward et al., 2001; Tate et al., 2003; Tate et al., 2004*b*). Potential co-dependence among meadows in the same management unit (allotment) will be investigated and modeled as random effects if required. Spatial correlation among meadows in close proximity will also be evaluated and modeled if required (Pinheiro and Bates 2000). Compliance with assumptions of normality and constant variance will be checked and variance and correlation structures for handling heteroscedasticity within the data as well as transformation of response variables applied if required. Analysis approaches will be refined in consultation with statisticians as the project progresses and the complete data structure is evident.

Strengths and Weaknesses

Strengths of this study design include: 1) examines a broad suite of grazing management factors; 2) does not depend upon implementation of grazing treatments over multiple years with associated logistical difficulties and risks; 3) can potentially yield management recommendations within 1 to 2 years; and 4) capitalizes upon the variability found across the population of interest, potentially increasing the scope on inference of the results. Weaknesses of this design include: 1) identifies and quantifies associations, cannot absolutely determine cause and effect; 2) requires significant data compilation and is vulnerable to unknown faults in existing data; and 3) variability may be so great that sample size requirements become infeasible with current resources.

Phase II

Treatment Definitions

The treatment in this phase of the study is cattle grazing. Three levels of cattle grazing will be examined in detail, and an additional 2-3 reference (long-term ungrazed) will also be monitored. Grazing treatment levels were derived from specific guidance provided by the Steering Committee and statistical requirements for a reference condition identified by the Design Team. Cattle grazing treatment levels are: 1) compliance with annual grazing standards and guidelines as defined in the most recent Sierra Nevada Forest Plan Amendment (USDA Forest Service 2004); 2) exclusion of cattle from wet areas of the meadow; 3) exclusion of cattle from the entire meadow; and 4) ungrazed by cattle for >15 years (reference condition). Baseline data will be collected in year 1 and grazing treatment levels 1 through 3 will be implemented and maintained during years 2 through 5 of this study by the study team. Grazing treatment level 4 is a reference condition which is already in existence and will be maintained throughout the course of the study. The use of the term "grazing treatments" in following sections refers to grazing management treatment levels 1 through 3, and the term "reference condition" refers to grazing management treatment level 4. The statistical importance of a reference condition treatment level is discussed below.

Approach

Phase II will be a randomized complete block design based upon collection of data before and after implementation of grazing treatments across 15 meadows (5 replicates of 3 grazing treatment levels), with the inclusion of 2-3 reference condition meadows to serve as a baselinc across the study period (17-18 meadows total). This phase will focus on meadows known to contain toads and which have low pack-stock use. Blocks are spatial clusters of 3 meadows. The primary purpose for clustering is to simplify formidable data collection logistics by having clusters of meadows in close (~10-20 km) proximity to each other. For statistical purposes, each treatment level will be represented in each cluster (1 replicate). Thus each cluster will have 3 meadows either currently or recently grazed under ambient grazing conditions. Grazing management prior to this phase will be quantified for each meadow for use as a covariate in analysis. Due to potential constrains imposed by location of ungrazed meadows, cluster location selection may not be completely random. Every effort will be made to introduce randomization in cluster site selection and to insure cluster site selection does not introduce bias towards one or more treatment levels. Depending upon project goals, cluster locations could be randomly selected in a stratified manner (*e.g.*, northern, central, southern range of the population; Stanislaus NF, Sierra NF). *After substantial reconnaissance efforts in 2005 and 2006, reference meadows (long-term ungrazed) of appropriate elevation and vegetation type could not be found in proximity to the study areas on the Sierra and Stanislaus National Forests. In 2007, two meadows in Yosemite National Park were added to fill the role of reference meadows and these will be monitored for the remainder of the study.*

Following a minimum of one year of baseline data collection on all 3 meadows in each cluster, grazing treatments (comply with annual grazing standards and guidelines, exclude cattle from wet portions of meadow, exclude cattle from entire meadow) will be randomly allocated to the 3 currently/recently grazed meadows within each cluster. Grazing treatments will be implemented annually during the remaining 4 years of the study, as will data collection across all 17-18 meadows.

Rationale for Baseline Data and Reference Condition

Grazing treatment level effect on toad populations, habitat, vegetation, etc. will be determined by comparison of relative changes in treated meadows to changes in ungrazed meadows over the study period (statistically significance/insignificant treatment level by year interaction). Collection of baseline data allows us to quantify initial differences between meadows scheduled for treatment implementation and reference condition meadows. Without baseline data, we cannot determine if any differences between treatment and reference meadows at the end of the study are due to grazing treatments or if these differences simply existed prior to grazing treatment implementation. For statistical purposes, the reference condition is the treatment level which is consistently applied across the entire study period allowing a reference or baseline condition against which to compare changes in treated meadows. Implementation of consistent management on a sub-set of experimental units across the study period reduces our vulnerability to confounding factors such as long or short term annual weather patterns. From a statistical perspective, a consistently applied heavy grazing treatment level could just as easily serve as a reference or baseline condition. From a biological and ecological standpoint, the ungrazed reference condition is of specific interest due to its potential to provide context for understanding the relative effects of cattle grazing.

Statistical Analysis

This phase of the study will generate data on a large number of dependent or response variables, requiring multiple analyses (development of statistical models for each response variable). Dependent variables available for analysis will be metrics of toad population (*e.g.*, # of toads by life stage, ratios of life stage abundances, density of breeding areas per meadow), habitat (*e.g.*, water temperature, aquatic plant cover), and vegetation (*e.g.*, species cover, diversity, structure). Independent variables will be grazing treatment which has 3 levels and year of which there will be five. Covariate data from each meadow will be available for a suite of static and dynamic factors (*e.g.*, lotic v. lentic, elevation, past grazing pressure, annual pack-stock use, annual snowfall). Reference meadow data will provide context, specifically related to background seasonal and annual variation in toad population sizes in ungrazed meadows. Several factors complicate the interpretation of this dataset and must be addressed directly within the analysis process. There will be co-dependence within the data due to repeated measures on each experimental unit (meadow) across a season and across years. Data from meadows within the same cluster will potentially be subject to spatial correlation. Based upon previous experience, we suspect that these data will not comply with the normal distribution nor will they have a constant variance. Finally, inherent variability between meadows due to non-cattle grazing related variable such as elevation, annual snowfall, geomorphology, etc. will almost certainly reduce or confound our ability to ascribe treatment effect. This will be addressed as much as possible by inclusion and modeling of these variables as covariates during statistical analysis.

We have had success in modeling similarly complex datasets using both linear mixed effects analysis and count-based regression models (Atwill et al., 2002; Tate et al., 2004*a*; Jackson et al., 2006). Linear mixed effects analysis is one feasible approach that will be explored in the analysis of this data (Pinheiro and Bates, 2000). Meadow identity and cluster will be treated as group effects to account for co-dependence introduced by repeated measures and spatial proximity, respectively. The model allows, if warranted, application of variance and correlation structures for handling heteroscedasticity within the data. Transformation of response variables will also be explored. We will also explore the application of negative binomial regression analysis and other count-based regression techniques, which we have found valuable in the analysis of data similar to that available in this study (*e.g.*, counts of toads). Software which allows for cluster or group variables (meadow identity and cluster) will be used (as explained in volume 2 of *Stata Statistical Software: Release 7.0*, *Reference H-P*, p. 530-534 [Stata Corporation, College Station, Tex.], 2001). Analysis approaches will be refined in consultation with statisticians as the project progresses and the complete data structure is evident. Preliminary analysis will be conducted on an annual basis to provide guidance for final analysis strategies, and as a standard quality control and assurance measure.

Strengths and Weaknesses

Strengths of Phase II study design include: 1) explicitly targets the three grazing treatment levels identified by the Steering Committee; 2) has potential to establish cause and effect, rather than identify associations/correlations; 3) is longitudinal, allowing examination of cumulative effects of treatment application over multiple years; 4) targets data collection efforts on a reasonable number of meadows allowing quantification of seasonal dynamics toad population, habitat, hydrologic, vegetation, etc. variables which may be important for interpreting study results. Weaknesses of the study design include: 1) treatment effects may take longer than 4 years of treatment implementation to become significant; 2) one year of baseline may be inadequate to accurately characterize initial conditions and differences between treatment and reference condition meadows; 3) the study is vulnerable to unforeseen confounding factors, and/or is under sampled to account for a large number of potential confounding factors; 4) the study is vulnerable to unforeseen logistical problems with grazing treatment implementation, and/or events such as prolonged drought, wild fire, etc.; 5) study results will likely not be available for incorporation into conservation strategies until 5 to 6 years after the study commences.

Study Meadow Selection

Phase I

As described above, this phase of the project primarily involves the use of existing data sources. Once a preliminary assessment and analysis of these sources and their applicability to our main questions has been completed, we will strategically select additional field sites for rapid assessment and/or return to field sites from existing data to gather data on missing variables of interest. Our goal will be to ensure that we have data from a gradient of livestock grazing levels and a gradient of Yosemite toad occupancy levels.

Phase II

Random Selection of Meadows/Allotments

All available distributional data on Yosemite toad was collected. Appendix A provides a list of the data sources considered for use in the analysis to determine sample sites. Each occurrence data point is associated with geographic coordinates (utm northing and easting) and/or a meadow. Because meadows are the sampling unit and not all Yosemite toad occurrences are associated with a meadow, all occurrences were overlaid in a Geographical Information System (GIS) environment with a meadows layer. Meadows that overlapped with the point occurrences are thought to have Yosemite Toad present. Only meadows with an elevation between 6000 and 10,000 feet and meadows in Stanislaus NF, Sierra NF and Yosemite NP are considered.

A randomized block design was utilized so that each block would contain four meadows which could later be assigned to each of the four grazing treatment types. The primary purpose for clustering the sample meadows into blocks is to simplify data collection logistics by having clusters of meadows in close proximity to each other (maximum of about 12 km apart). A secondary benefit from blocking is that all meadows within the block should have similar environmental conditions, such as temperature and precipitation.

Each block, which was actually a hexagon, had an area of 100 km^2 (Fig. 1). The hexagons were overlaid with the distribution of the Yosemite toad. Hexagons containing at least five meadows occupied by Yosemite toad ($n = 27$) were assigned random numbers and arranged in order from lowest number to highest number (Fig. 2). At least one meadow in the block must not have been grazed by cattle for at least the previous 15 years. Meadows adjacent (within 2km) to the block were included in the list of potential sample meadows to provide options for this ungrazed category.

For initial meadow selection, we reviewed the blocks in their assigned random order and assessed whether they met minimal qualitative criteria for final selection. These criteria included: presence of relatively robust toad populations (e.g. multiple life stages, multiple breeding sites, relatively population sizes) and accessibility (distances to drivable roads, etc). If a block did not meet these criteria, then the next block was assessed for inclusion into the study. In addition, we imposed a criterion to ensure representation of study meadows on both National Forests. Because more blocks were initially present on the Sierra than the Stanislaus National Forest, we required that minimum of two blocks be chosen from the Stanislaus National Forest. The selected blocks were associated with 8 livestock grazing allotments; they are presented here in the random order in which they were chosen: Dinkey, Collins, Patterson Mountain, Highland

Lakes, Blasingame, Kaiser, Mt. Tom, and Herring Creek. These allotments thus became our focal set. Even though we ultimately need only 5 allotments to conduct the study (3 meadows x 5 allotments = 15 study meadows + 2-3 reference meadows), we will evaluate all 8 in the next step of site selection to allow some flexibility. In addition, we plan to collect baseline data on more than 15 meadows, and assess their general condition. Meadows that are extreme outliers may be eliminated during the later years of the study which should increase our ability to provide more reliable and precise results to managers.

Final Meadow Selection

To aid in final study meadow selection, we sent a questionnaire to Forest staff to gather the following information on all meadows in these 8 allotments: proximity to ungrazed meadows, detailed population data on Yosemite toads (number of breeding sites, multiple years, abundance by life stage, etc.), potential logistical difficulties, road access and condition, current packstock and recreational (hiking, camping) use, types of livestock controls employed (e.g. fencing, herding), availability of records on history of livestock use, and availability of quantitative information on meeting standards and meadow/stream conditions, occurrence of meadow restoration activities, and rodent densities/control measures (Appendix B).

Based on information provided to the design team via the questionnaire, logistical constraints (i.e., accessibility and the need for clusters of 3 meadows for sampling efficiency), and field visits in spring 2005, we reduced the set of 8 allotments to 5: Dinkey, Patterson Mountain, and Blasingame on the Sierra National Forest and Highland Lakes and Herring Creek on the Stanislaus National Forest.

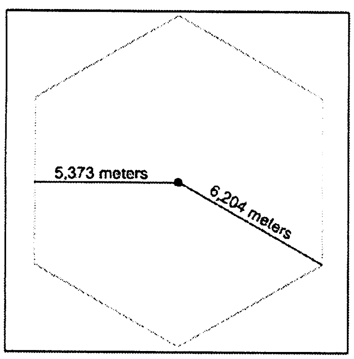

Figure 1. Depiction of the 100 km^2 hexagon.

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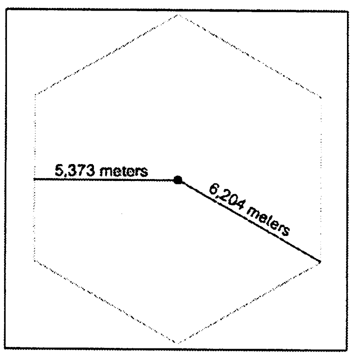

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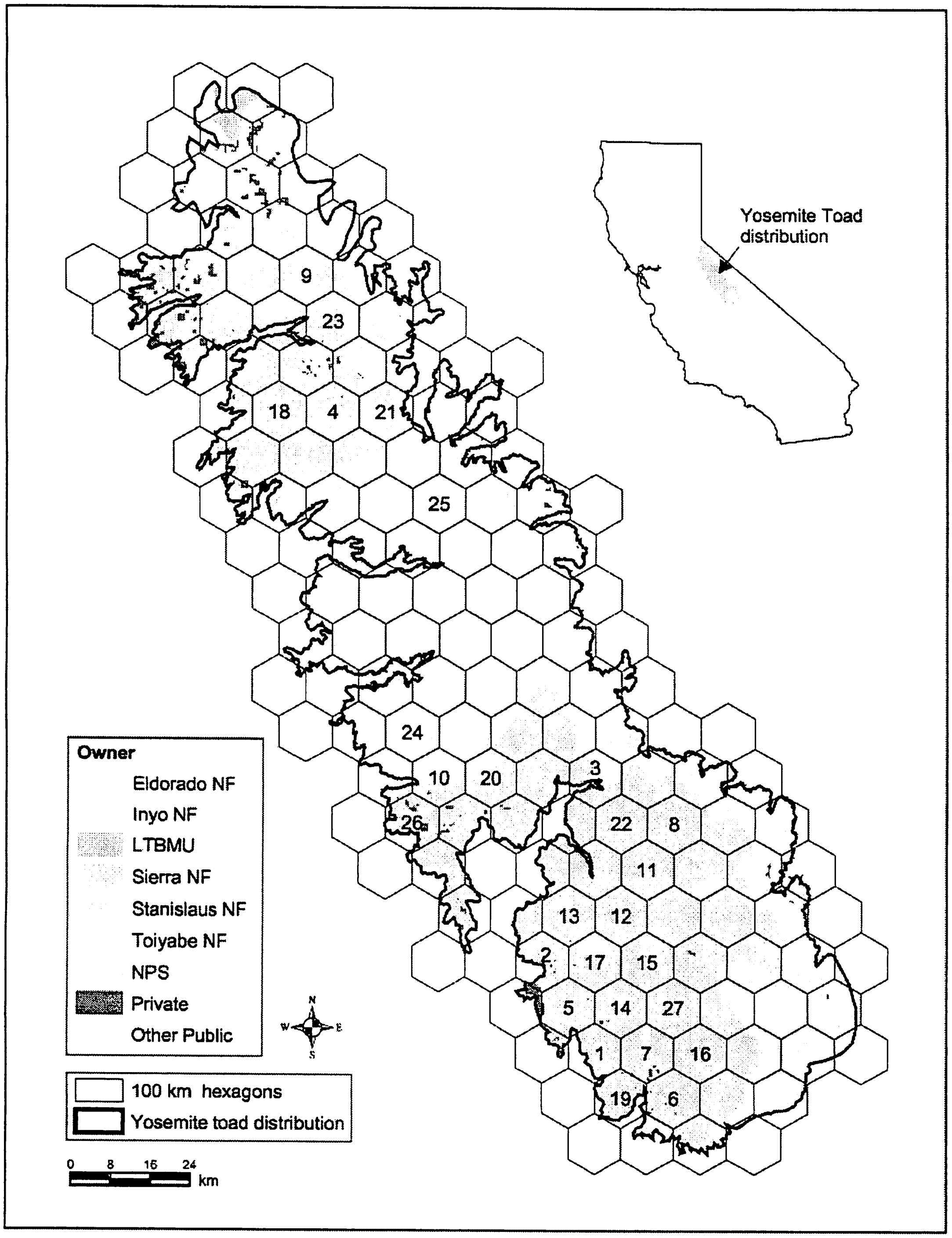

Figure 2. Yosemite toad distribution with randomized block order.

Data Collection Methods

Yosemite Toad Populations and Microhabitat

Determination of Population Attributes/Parameters

To develop reliable estimates of the effects of livestock grazing on Yosemite toad populations, we will need to use repeatable sampling methods that will ideally provide information on relative abundances or densities of all life stages. Yosemite toads have a relatively short breeding season; up to 5 weeks, but at some locations as few as 1-2 weeks. Research and anecdotal accounts indicate that adults are not easy to detect outside of this season as they may be active primarily at night and take refuge in rodent burrows (USDI Fish and Wildlife Service 2002, Sadinsky 2004). Thus the timing and intensity of our sampling will dictate the potential methods we will use.

Phase I – For analysis of existing data, we will rely on toad occupancy, breeding status, and potentially indices of abundance as response variables. Because these data will be from a variety of sources, we are developing metrics that are consistent among the sources and performing a "validation" of data by comparing multiple sources for the same meadow locations. For example, extensive surveys for Yosemite toads have been conducted on the Sierra National Forest. Prior to using these data for analyses of occupancy status, we will compare ("validate") the occupancy status to data collected by the Sierra Nevada Amphibian Monitoring Team (C. Brown, pers. comm.) and other researchers (R. Grasso, pers. comm.) at the same sites.

Similarly for any additional meadows where new field data are collected, we will use metrics that can be rapidly recorded and converted to the same indices used for the existing data analysis. These metrics also need to be somewhat consistent over the timeframe of the field work since different life stages will occur at different relative abundances depending on the timing of sampling (e.g., early vs. late summer). However, because occupancy is the key toad population parameter here, the timing of this work is less critical. For example, we could use a metric that represented the number of breeding/rearing areas which could be determined through observation of eggs, larvae, or early metamorphic toads.

Phase II – The overall study design for this phase includes only five replicate meadows per grazing treatment, so identifying population attributes that will provide appropriate sensitivity in the face of the expected high levels of variability is critical. We will use a combination of abundance data, capture-recapture population estimates, and indices of breeding/rearing areas to characterize toad populations. Detailed information on relative abundance by sex and life stage (eggs, larvae, metamorphic toads [young of the year], subadults, adults) collected during the baseline year will provide insights into ambient levels of variability in toad populations and guidance on selection of meadows. In subsequent years of the study, we will collect more detailed information on abundance, including estimates of population size by life stage using capture-recapture techniques. We will make an effort to collect information on all life stages, though adult and subadult (1+ year olds) toads present challenges because of their limited activity periods at meadows. Following assessment of data from the baseline year, we will adjust the timing of sampling as needed to increase likelihood of encountering adults at least a subset of our sites (e.g. at all meadows within 2 blocks/allotments).

In addition, we will record information on body size/weight and evidence of disease for a subset of individual larvae and toads to develop a description of the health of each population. We are currently working with researchers in the Briggs Lab at U.C. Berkeley to evaluate levels of chytrid fungus (*Batrachochytrium*) presence (and prevalence) in our study populations (C.

Briggs, pers. comm.). To determine if we will have the power to detect differences in grazing treatments at biologically appropriate levels if they exist, we will conduct sample size analyses and analyze sources of variability from existing data. We will use data on intra and inter-annual toad abundance gathered by the Sierra Nevada Monitoring Team (C. Brown, pers. comm.). Baseline data collected in the first year of our study will provide additional information so that sample sizes or sub-sampling approaches can be modified to improve estimates of and confidence in effect size detection.

Microhabitat associations for individual toads and for groups of eggs or larvae will also be measured and will include attributes that characterize surrounding immediate (< 1m) aquatic environments, such as water depth, vegetation, etc. This information, when analyzed in combination with the meadow and pool habitat data, will give insights on the specific features of the environment that are utilized by and important to different life stages of toads. The potential effects of cattle grazing on these features can then be assessed.

Field Methods

The observational component of Phase I is dependent on the inclusion of a large number of meadows along a gradient of conditions, so we will use metrics of toad populations that can be collected rapidly in the field. Presence/absence of toads and counts of breeding/rearing areas are two such metrics (Thompson et al. 1998, Knapp et al. 2003). Phase I meadows will be visited only once during the study so we will use techniques that have a high likelihood of detecting toads if they are there. Visual encounter surveys and dip-netting (Heyer et al. 1994) will be the primary methods used to detect toads and these surveys will focus on areas of the meadow where toads are most likely to occur (e.g. pooled water) to increase efficiency. Depending on the timing of sampling, we should encounter either or both larval and metamorphic life stages so we will also be able to quantify the density of breeding/rearing areas. Counts of all life stages will be made so that it will be possible to develop abundance indices, though differential timing of sampling may ultimately confound our ability to generate reliable estimates of abundances by life stage.

For Phase II, our sampling approaches need to generate precise estimates of toad population sizes. The following are potential metrics: 1) abundance of toads by life stage (eggs, larvae, metamorphic toads, sub-adults, adults), 2) ratios of life stage abundances, especially as an index of survival from larval to metamorphic stages, 3) population size and survival rates of toads can be calculated (if capture-recapture techniques can be used successfully), and 4) density of breeding/rearing areas per meadow. Because of life history of the toad and limited access to meadows under snow, we will likely encounter primarily larvae and metamorphic toads in the study meadows. These life stages are notoriously difficult for estimates of population size because their numbers can vary dramatically within meadows, among meadows, and among years (e.g. Jung et al. 2002). We will deal with this in several ways: 1) by choosing sub-sites within meadows and doing multiple counts of all life stages within sub-sites over the course of a day or two (counts of the same area by different observers may area also being tested to increase precision of estimates), 2) using a temporary mark on larvae for sub-sites within meadows and returning within a day to count marked and unmarked larvae, and 3) visiting each meadow in two different time periods so that counts of larvae are made during the first time period and counts of metamorphic toads are made during the second time period, which should provide a rough estimate of survival from larval stage to metamorphosis. We will also attempt to survey a subset of meadows early in the season so that counts and population estimates of subadult and adult toads (using capture-recapture techniques) can be made. Finally, we will also utilize information on the density and occupancy of breeding/rearing areas as an index of toad population size. Draft protocols for toad population data collection can be found in Appendices C1-C3.

Microhabitat data will be collected on groups of eggs or larvae. Variables representing immediate habitat conditions in the wet areas of the meadow will be the main focus including: water depth, substrate, presence of emergent vegetation, etc. (Figure 3). In addition, habitat characteristics that are believed to represent features important to terrestrial life stages of toads (subadults and adults) will be quantified, especially burrow abundance within and immediately adjacent to meadows and connectivity to upland springs/streams. Draft protocols for microhabitat data collection can be found in Appendices C1-C3.

Current and Past Cattle Grazing Management

Cattle grazing management (current and past) will be quantified from: 1) review of allotment management plans; 2) standardized survey of USFS range management staff about grazing management practices and observed grazing intensity for each allotment and meadow enrolled in the survey; and 3) standardized survey of on-the-ground cattle managers for each permitted allotment and meadow enrolled in study 1 and 2. We will use a standardized grazing management survey-questionnaire which has been specifically designed and extensively used to quantify both current and past grazing management on meadows (Ward et al. 2001 – over 150 interviews with grazing managers on both public and private rangelands). The survey allows us to quantify standard grazing management practices such as number of cattle, duration of grazing, season of grazing, frequency of grazing, and implementation of cattle distribution practices such as herding and placement of off-site supplemental feeds for each meadow enrolled in the survey. Questions designed to quantify meadow use by packstock will be incorporated into the questionnaire for this project. Our model for obtaining detailed management information from individual managers is to work closely with county-based UC Cooperative Extension advisors, the California Cattlemen's Association, and the California Farm Bureau Federation to make individual managers aware of objectives of the project, it's potential to aid in science-based resolution of critical resource management issues, and the importance of their participation and assistance in collecting objective and accurate data (Lewis et al., 2001; Ward et al., 2001; Lewis et al., 2005).

Hydrologic and Water Quality Data

Hydrologic and water quality related habitat variables to be monitored at each study site include: water temperature, pool habitat volume (area and depth of pools), water table dynamics and connectivity of pools to meadow water table dynamics, flow rate and volume for lotic systems, dissolved oxygen, pH, conductivity, and turbidity in pools (Figure 3). Data on these parameters will be collected bi-weekly during May/June through August/September snow and weather permitting. Water temperature in pools in each meadow will be collected via placement of automatic temperature loggers set to record temperature (Onset Computer Corp. Optic StowAway) on a 0.5 hour time step, allowing for characterization and analysis of a suite of temperature metrics (Tate et al., 2005*b*). Air temperature will be collected in each meadow as a covariate (Tate et al., 2005*c*). Pool surface area and depth will be hand measured relative to bench marks for consistency from sample period to sample period. Two inch wells will be dug to bedrock or clay-stone layer and lined with PVC pipe to allow monitoring of water table depth.

Wells will be set out in a grid of transects perpendicular to and transecting pools to facilitate correlation of pool volume to water table dynamics (*e.g.*, connectivity). Naturally conservative tracer (*e.g.*, Cl, Br) injection and recovery techniques may be employed to further characterize hydrologic connectivity in lentic systems and hydrologic residence times in lotic systems (stream associated). Flow rate and volume in and out of lotic meadows will be measured by hand using the area-velocity method at the top and bottom of each meadow (instream). Dissolved oxygen levels, conductivity (multi-parameter YSI meter) and turbidity (Orbeco-Hellige portable turbidity meter) will be determined in the field. All equipment (water quality meters, flow meters, temperature loggers, etc.) will be calibrated and used according to manufacture's instructions. Initial and follow-up training of field crew in operation of equipment and collection of field data will be conducted and documented.

Vegetation

Vegetation and related grazing intensity variables to be collected in this study include: plant species composition and cover, residual dry matter, stubble height, and above ground biomass (Figure 3). Vegetation parameters will be collected in conjunction with piezometer wells as described in the Hydrologic and Water Quality section above since species composition and productivity are related to water table hydrology (Allen-Diaz 1991). The wells will be laid out in transects perpendicular to and transecting meadow pools. Within a 3 m radius of each well, we will randomly place a livestock exclusion cage. The number of cages will be determined in conjunction with the number of piezometer wells, and cages will not be placed in pools. A paired, uncaged plot, will be located and marked with a wooden redwood stake at the same time that the cage is installed. A modified 10-pt frame (collecting a total of 100 points) will be used to determine species composition within the cage. Then the biomass inside the cage will be clipped (using a 25x25 cm square quadrat) as close to peak standing crop as possible in order to determine above-ground biomass (grams per $1/16^{th}$ m^2). At the uncaged plot, stubble height will be recorded from 5 points inside the $1/16^{th}$ m^2 quadrat using a centimeter stick, and biomass will be clipped at the same time in order to determine utilization. Periodic random sampling of grazed meadow biomass will be conducting during the season to track stubble height S&Gs. Cages will be built of woven wire, with a 1 m^2 base, narrowing to 50 cm^2 at the top and be approximately 5 ft tall. Residual dry matter and end-of-season stubble height will be measured at a random location within the 3 meter radius of each piezometer well in September when cages will be collected for the season. The cages will be moved to a new random location near the piezometer well the following spring to remove any cage effects.

Again using the random piezometer transects as the focal point for vegetation data collection, we will collect vegetation species information by functional group at 10 cm intervals along a line point transect placed adjacent to the piezometer transect in order to examine meadow vegetation gradients. At this time we propose to use grass, sedge, rush, forb, shrub and semi-shrub as functional groups. Water, rock, hoof print, small mammal burrows, litter, and manure will also be recorded. Vegetation data will be collected and recorded in a spatially explicit fashion so that relationships between and among hydrologic and vegetation variables can be examined using gradient analysis and spatial statistics.

One time environmental variables include: slope, aspect, and elevation will be recorded using standard field instruments at each meadow. At each piezometer well within the meadow, slope, aspect and soil profile information will be recorded. Soil samples will be collected from each 10 cm interval along the soil core when the piezometer well holes are dug. Soil samples will be placed in sealed plastic bags, placed in a cooler, brought back to the lab and frozen for later analysis.

Integration of Toad Population, Habitat and Livestock Grazing Data

The success of this study will ultimately depend on the integration and analysis of a data covering a diverse set of variables collected at several spatial scales. Table 1 provides a conceptual approach to that integration and Figure 3 shows a schematic of field data collection. The most extensive scale represents the context for each study meadow and will be based on a hydrologically defined basin and/or whole grazing allotments. Data at this scale will be derived primarily through GIS and include information such as elevation, number of occupied meadows surrounding study meadow, etc. Some of these data have already been gathered to aid in study meadow selection and we will continue to refine and add to this as needed. The next less extensive scale is that of the whole meadow which reflects general vegetative and hydrologic conditions, the distribution of occupied and potential breeding sites, and overall livestock use information. Within a given meadow we expect to find multiple discreet or definable wet areas that could be used for breeding/rearing of toads and toad larvae. At this scale, we will characterize the environmental context, identify the habitats available to toads, and document finer scale effects of livestock on these environments. Finally, we will also collect data on larval groups and individual toads, including estimates of abundance of larvae and metamorphic toads, descriptions of local microhabitats, and evidence for direct effects of livestock on these life stages. Habitat preferences of toads will then be assessed through comparison with data gathered on available habitats/environmental context.

Table 1. Scales of interest and potential field methods for Yosemite toads, their habitat, and livestock use. Scales (underlined text, e.g. "whole meadow") are ordered from most extensive to most local. Field methods should be considered preliminary; more details are provided in sampling protocols (Appendices C1-C3).

TOADS	TOAD HABITAT	LIVESTOCK USE	FIELD METHODS
Allotment/Basin/Multi-Meadow (Context)			
number of populations, proportion of meadows/area occupied	GIS generated environmental variables such as: elevational range, climate regime / history, topographic features	allotment-wide use, distribution over time	- Forest GIS data on toad populations and meadows - Information from Forest range conservationists and permittees (livestock)
distribution of occupied and unoccupied meadows	spatial distribution of suitable meadows, connectivity among meadows		
Whole Meadow			
number of breeding/rearing areas	spatial distribution of breeding/rearing areas and other wet areas of meadow - e.g. area of aquatic habitats by type	cattle use levels /disturbance of all breeding/rearing areas and other wet areas of meadow	cross meadow transects or grids and point intercept data ("permanent")
movement/dispersal corridors – e.g. hydrologic connectivity to other meadows/upland springs, overland distances to upland springs	number/size/length? of streams or moist riparian areas	cattle use of riparian corridors	
	area of meadow	pattern of cattle use of whole meadow	
	meadow hydrologic patterns vegetation composition/structure in wet and dry areas		
	slope/aspect of meadow		
	predators/competitors?		
	other disturbance – e.g. recreation, packstock		
Suitable Breeding/Rearing Areas (occupied and unoccupied for comparison)			
population size (and survival rates?) by life stage per breeding area	characteristics of breeding/rearing areas - e.g. water depth, temperature, vegetation, substrate, water quality	cattle effects on breeding/rearing areas – e.g. chiseling?, hoof punches, water quality (cow pies?)	collection of more intensive data – via stations, short transects, or area based estimates, capture-recapture of terrestrial toad life stages
	predators/competitors – snakes, birds, other amphibians		
Individual Aquatic Toads, Egg Masses, Larval Groups			
health – e.g. disease, length/weight ratios.	microhabitat associations of individual toads, metamorphic toads, egg masses, larvae groups - e.g. associated substrate, water temps, water quality	evidence for direct effects on toads - e.g. trampling, stranding of eggs, larvae in hoof prints.	visual inspection / local scale measurements, capture-recapture and/or repeated counts of aquatic toad life stages